Theor Appl Genet (2005) 110: 706–713
DOI 10.1007/s00122-004-1896-1

ORIGINAL PAPER

E. Ortiz-Vázquez · D. Kaemmer · H-B. Zhang
J. Muth · M. Rodríguez-Mendiola · C. Arias-Castro
Andrew James

Construction and characterization of a plant transformation-competent BIBAC library of the black Sigatoka-resistant banana *Musa acuminata* cv. Tuu Gia (AA)

Received: 4 August 2004 / Accepted: 24 November 2004 / Published online: 14 January 2005

Abstract A plant transformation-competent binary bacterial artificial chromosome (BIBAC) library was constructed from *Musa acuminata* cv. Tuu Gia (AA), a black Sigatoka-resistant diploid banana. After digestion of high-molecular-weight banana DNA by *Hin*dIII, several methods of DNA size selection were tested, followed by ligation, using a vector/insert molar ratio of 4:1. The library consists of 30,700 clones stored in 80 384-well microtiter plates. The mean insert size was estimated to be 100 kb, and the frequency of inserts with internal *Not*I sites was 61%. The majority of insert sizes fell into the range of 100 ± 20 kb, making them suitable for *Agrobacterium*-mediated transformation. Only 1% and 0.9% of the clones contain chloroplast and mitochondrial DNA, respectively. This is the first BIBAC library for banana, estimated to represent five times its haploid genome (600 Mbp). It was demonstrated by hybridization that the library contains typical members of resistance gene and defense gene families that can be used for transformation of disease susceptible banana cultivars for banana genetic improvement.

Communicated by R. Hagemann

E. Ortiz-Vázquez · D. Kaemmer · A. James
Centro de Investigación Científica de Yucatán,
Mérida, Yucatán, México

E. Ortiz-Vázquez · M. Rodríguez-Mendiola · C. Arias-Castro
Centro de Investigación y Graduados Agropecuarios,
ITA No. 26, Tlajomulco de Zúñiga, Jalisco, México

H-B. Zhang
Department of Soils and Crop Science, Texas A&M University,
College Station, TX 77843, USA

J. Muth
Fraunhofer Institute, 52074 Aachen, Germany

D. Kaemmer
PHARMAPLANT Arznei- und Gewürzpflanzen,
Forshungs- und Saatzucht, Artern, Germany

E. Ortiz-Vázquez (✉)
Instituto Tecnológico de Mérida,
Av. Tecnológico S/N C.P., 97118 Mérida,
Yucatán, México
E-mail: eortiz@itmerida.mx
Fax: +52-9999-448479

Introduction

Banana and plantain are grown in almost 120 countries of tropical and subtropical regions, producing about 100 million tons of fruit every year. In the context of production value, bananas, and plantains are the fourth worldwide crop after rice, wheat, and maize (INIBAP 2001). However, the global production of bananas and plantains is being threatened by the foliar fungal pathogen *Mycosphaerella fijiensis*, the causative agent of black Sigatoka, or black leaf streak disease (BLS), which can lead to reductions in yield of more than 50%. Banana and plantain are clonally propagated monocotyledonous plants that are judged recalcitrant to conventional breeding, chiefly due to high levels of sterility, differing levels of ploidy, and the necessity of producing parthenocarpic progeny. An alternative solution to this problem is the application of genetic transformation for existing commercial cultivars.

Musa acuminata cv. Tuu Gia (AA), an edible diploid banana, is thought to have originated in Vietnam (I. Buddenhagen, personal communication), and according to the chloroplast and mitochondrial RFLP analysis, this parthenocarpic accession is considered to have a cytotype paternally related with *M. acuminata* spp. *errans* clone (Carreel et al. 2002). It is recognized as having durable and high resistance to *M. fijiensis* (Fullerton and Olsen 1995; Ortiz 2000). In this study, we present the results of the construction and characterization of a binary bacterial artificial chromosome (BIBAC) genomic library of *M. acuminata* cv. Tuu Gia (AA). The BIBAC technology can be used as a means to obtain transformation-ready, genomic libraries with

large inserts, enabling the transfer of gene clusters up to 120 kb into plant cells (Hamilton et al. 1996, 1999; Liu et al. 1999, 2002; He et al. 2003). Although studies had demonstrated that the cultivar Tuu Gia is able to produce viable pollen (Perea-Dallos 1998), Tuu Gia is considered sterile; therefore, genomic applications that rely on genetic mapping, such as map-based cloning, could not be applied. However, using other approaches for gene or gene cluster discovery, such as the use of resistance and defense gene analogues as probes, are some of the multiple applications. Access to a transformation-ready large-insert genomic library of Tuu Gia will allow the study of the function of various genes, especially those coding for proteins involved in agronomical important traits, such as disease resistance.

Deciphering the banana genome is the task of the International *Musa* Genomics Consortium, for which the BIBAC library of Tuu Gia will be very useful in parallel with already available BAC libraries of *M. acuminata* Colla type Calcutta IV (AA) and *M. balbisiana* (BB).

Materials and methods

Plant materials

The youngest fully developed leaves were collected from greenhouse-grown ex vitro plants of *M. acuminata* cv. Tuu Gia (AA) obtained from the INIBAP *Musa* Germplasm Transit Centre in Leuven, Belgium (accession no. ITC 610), which had been previously kept in darkness for 48 h to reduce starch levels (P. Gresshoff, personal communication).

Preparation of BIBAC vector

Escherichia coli strain DH10B containing the BIBAC vector pCLD04541 was grown in LB medium (Sigma, USA) augmented with tetracycline (15 mg/l) at 37°C and 250 rpm overnight. The DNA was prepared by the alkaline lysis method, followed by two rounds of cesium chloride gradient purification (Sambrook and Russell 2001). The vector was digested with *Hin*dIII to completion and dephosphorylated to prevent self-ligation.

Preparation of electrocompetent cells

This procedure was carried out according to Sambrook and Russell (2001), with some modifications. SOC medium without magnesium (2% tryptone, 0.5% yeast extract, 10 mM KCl, 20 mM glucose, pH 7.0) was inoculated using a fresh colony of DH10B cells and grown at 37°C for 4–5 h until $OD_{550} = 0.7$. After the washing steps, the cells were finally resuspended in the minimum amount of 1 ml ice-cold washing buffer; aliquots of 20 µl-stocks were frozen using an ethanol/dry ice bath and stored at −80°C.

Preparation of high-molecular-weight DNA

Nuclei were isolated according to Zhang et al. (1995, 1996a), with some modifications. One hundred grams of the youngest banana leaves were ground into a fine powder in liquid nitrogen and the powder transferred into an ice-cold 1,000-ml beaker containing 1× homogenization buffer (0.01 M Trizma base, 0.08 M KCl, 0.01 M EDTA, 1 mM spermidine, 1 mM spermine, 0.5 M sucrose, pH 9.4–9.45) plus 0.15% β-mercaptoethanol, 0.5% Triton X-100 and 2% polyvinylpyrolidone (hereafter known as washing buffer). The homogenate was filtered through two layers of cheesecloth and one layer of Miracloth (Calbiochem, USA), and the nuclei were recovered by centrifugation at 1,800 *g* at 4°C for 5 min. Nuclei were then resuspended in washing buffer, centrifuged at 57 *g* for 2 min, and the supernatant containing the nuclei finally filtered using a layer of Miracloth, followed by four additional washing steps, including centrifugation at 1,800 *g* and 4°C for 15 min. Nuclei were again resuspended in 1 ml 1× homogenization buffer, embedded in LMP agarose plugs (0.5% final concentration), and disposed in 80-µl molds. A subsequent digestion of plugs with lysis buffer (0.5 M EDTA pH 9.3, 1% sodium lauryl sarcosine, 0.2 mg/ml proteinase K) was carried out for 48 h at 50°C. Three washes were performed in ice-cold TE (10 mM Tris-HCl, pH 8.0; 1 mM EDTA, pH 8.0) plus 0.1 mM phenylmethyl sulfonyl fluoride (PMSF), and then three washes in ice-cold TE without PMSF, with 1 h for each wash, followed by pulse-field-electrophoresis (PFGE), as conditioned by Tao et al. (2002), to eliminate small fragments, and finally stored in TE at 4°C.

BIBAC library construction

Agarose plugs previously cut into 24 pieces to improve enzyme diffusion were incubated in 1 ml incubation buffer (50 mM Tris-HCl, 10 mM $MgCl_2$, 50 mM NaCl, 2 mM spermidine, 1 mM DTT) on ice for 2 h, with buffer exchange after 1 h. Each lot of eight pieces of plugs was transferred to different microcentrifuge tubes for partial digestion with 170 µl digestion buffer (incubation buffer, 0.5 mg/ml BSA) plus *Hin*dIII enzyme (Invitrogen, USA), using a range of 0–4.8 U/tube. The digestion reaction was incubated on ice for 90 min and a further 8 min at 37°C in a water bath. The reaction was stopped by adding one tenth volume 0.5 M EDTA, pH 8.0. Partially digested DNA in the plugs was analyzed by PFGE, following conditions reported by Tao et al. (2002), to determine partial digestion conditions that produced the majority of restricted fragments in a range from 100 kb to 300 kb.

According to partial digestion conditions determined above, large-scale partial digestion was performed using ten plugs, which were digested using 1.2, 1.4, and 1.6 U/tube of *Hin*dIII and loaded in a 1% preparative agarose gel in 0.5× TBE buffer. Restriction fragments were resolved in a CHEF DRII System (Bio-Rad, USA) under the following conditions: 6 V/cm, 90 s switch time, 12.5°C, 0.5×TBE, for 14 h, followed by 1–6 s switch time, 6 V/cm, 12.5°C, for 6 h. Marker lanes were excised from the gel, stained with ethidium bromide, and realigned to the unstained portion of the gel. The gel section containing fragments of 100–300 kb was cut in two pieces of 5.5 mm each and electroeluted using dialysis tubes with an exclusion molecular weight of 14 kDa (Sigma) by PFGE at 6 V/cm, 40 s switch time, 12.5°C, in 4 mM Tris-acetate, 1 mM EDTA, for 4 h. The concentration of DNA fragments was estimated by standard agarose gel electrophoresis, using lambda DNA of known concentration as a reference.

Several ligations were performed at different molar ratios of vector to the insert (1:1, 2:1, 4:1). Ligation mixtures contained 8.5 Weiss U/ng total DNA (insert + vector) of T4 DNA Ligase (New England BioLabs, USA) and were incubated for 12 h at 16°C.

Subsequently, 1.5 µl ligation mixture was used to transform *E. coli* DH10B competent cells by electroporation in a Gibco–BRL Cell Porator and Voltage Booster System (Gibco–BRL, Grand Island, N.Y., USA) at 350 V, 330 μF capacitance, low Ω impedance, fast charge rate, 4 kΩ resistance; and immediately transferred into 1 ml SOC medium (2% Bactotryptone, 0.5% Bacto yeast extract, 10 mM KCl, 10 mM $MgCl_2$, 10 mM $MgSO_4$, 20 mM glucose, pH 7.0); incubated for 1 h at 37°C and 250 rpm; and then plated on selective medium (LB + 1.4 mg/l IPTG + 60 mg/l X-Gal + 15 mg/l tetracycline). White colonies were randomly selected for plasmid preparation (Sambrook and Russell 2001). Plasmids were digested with 1 U *Not*I in 40-µl reactions. The mean insert size was estimated by PFGE at 6 V/cm, 5–15 s switch time, 12.5°C, for 13.5 h.

White colonies from the best ligation were inoculated into 384-well plates containing 60 µl LB freezing medium [LB, 36 mM K_2HPO_4, 13.2 mM KH_2PO_4, 1.7 mM sodium citrate, 0.4 mM $MgSO_4$, 6.8 mM $(NH_4)_2SO_4$, 4.4% glycerol] with 15 mg/l tetracycline and incubated at 37°C overnight. Finally, the plates were stored at −80°C.

BIBAC library screening

The BIBAC library was robotically double-spotted onto 22.5×22.5-cm Hybond-N^+ nylon membranes (Amersham, Pharmacia Biotech, USA) in a 3×3 format, each containing 1,536 clones, using the QPixII robot (Genetix, Aachen, Germany).

The characterization of the library included the estimation of the mean insert size and insert size distribution as well as the number of clones containing repetitive and organellar DNA, using total genomic DNA, a chloroplast DNA mix (*ndhA*, *rbcL*, and *psbA* genes from barley), and mitochondrial DNA fragments (*nad4*, *atp6*, and *atp9* genes from soybean), respectively, as probes.

Two EST clones (TG-BR and HEM) obtained by differential display from wounded tissue of *M. acuminata* cv. Tuu Gia (AA) treated with *M. fijiensis* extracts (INCO-DC project IC18-CT97-0192; C. Navarro et al. unpublished) and a single PCR fragment amplified from Tuu Gia DNA, using NBS-LRR RGA9 primers from rice (5-CACGACTCTTGCTCAAATGG-3; 5-GCCTCCACATATTCCCACAA-3), were also used to demonstrate the usefulness of the library for gene fishing. The preparation of probes was performed by radioactive labeling, using [^{35}S]-dATP, employing random priming. After hybridization, membranes were treated according to Sambrook and Russell (2001) and immediately exposed to a Kodak BioMax MR film for 2–8 days. In order to confirm positive BIBAC clones, they were grown for plasmid preparation (Sambrook and Russell 2001), digested with *Not*I, analyzed by PFGE, and finally Southern-transferred. The Southern membrane was separately hybridized against EST-TGBR and TG-RGC9 probes.

Results

Construction of the BIBAC library

A large-insert arrayed BIBAC library was constructed from *Hin*dIII partially digested nuclear DNA of *M. acuminata* cv. Tuu Gia (AA) in the binary vector pCLD04541 at a 4:1 vector:insert molar ratio and with a single size selection. Transformation efficiency was estimated to have an average of 1.75×10^5 clones/µg of DNA, using self-made electrocompetent cells. A total of 30,700 clones were arrayed in 80 384-well plates (20 empty wells were counted).

Characterization of the BIBAC library

To estimate the insert size of the library, we randomly selected 120 clones, which were digested with *Not*I and analyzed by PFGE. The results showed that the library had a mean insert size of 100 kb. The frequency of inserts with internal *Not*I sites was 61% (Fig. 1). The majority of the insert sizes fell into a range of 100–120 kb, with more than 50% of inserts larger than 100 kb (Fig. 2). No insert-empty clones were observed from the 120 random clones analyzed. Five different BIBAC clones were tested for stability analysis and showed identical *Not*I-digested restriction fragment patterns from generations 1–100 in *E. coli*. (data not shown).

A total of five filters (7,680 clones) representing 25% of the library were screened with organellar DNA; 1% and 0.9% of the library clones were identified to contain

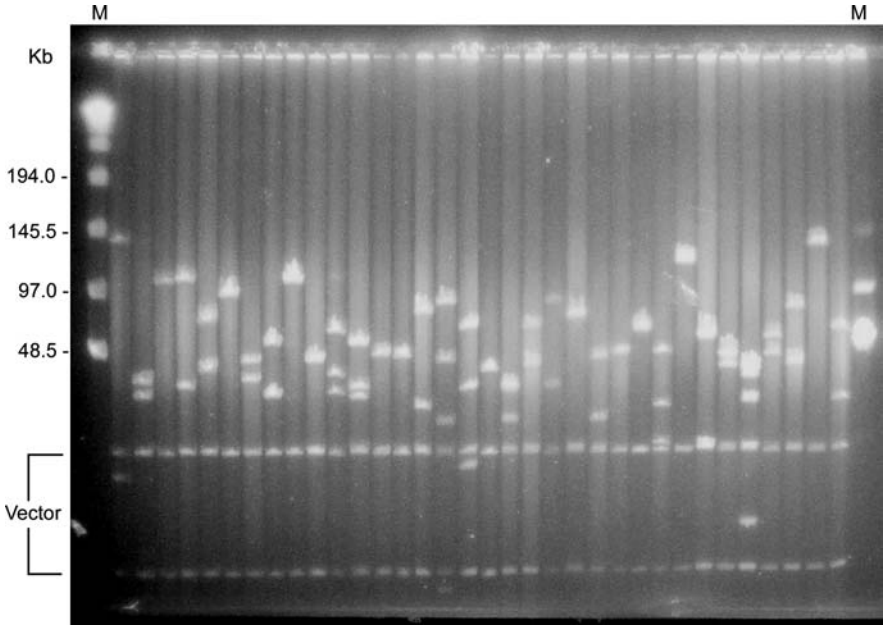

Fig. 1 Analysis of 30 random binary bacterial artificial chromosome (BIBAC) clones from the banana BIBAC library, digested with *Not*I and separated by pulse-field-electrophoresis (PFGE). *Lanes 1 and 36*, indicated by letter *M*, are lambda-ladder PFGE markers and the *remaining lanes* are BIBACs. The insert size of each BIBAC clone was estimated by adding up all insert bands of the clone, using the lambda-ladder markers as the reference

chloroplast and mitochondrial DNA sequences, respectively (Fig. 3). When total genomic DNA of *M. acuminata* cv. Tuu Gia (AA) was used as a probe, hybridization signals of different intensities were obtained for different clones, indicating that highly repetitive DNA (strong signals) was present in an average of 27% of 4,608 randomly selected clones (data not shown).

Taking into account that the size of the haploid banana genome is estimated to be 600 Mb (Dolezel et al. 1994), this BIBAC library represents 5.1 times the haploid genome, after subtracting 584 clones of organellar DNA. According to Clarke and Carbon (1976) the probability of obtaining a particular clone, using a single-copy sequence probe, is greater than 99%.

When 4,608 clones (three filters) of the library were screened with the EST sequences, 65 positive clones were obtained for the EST-TGBR probe (Fig. 4) and 38 positive clones for the EST-HEM probe. Both probes were obtained using differential display after the Tuu Gia

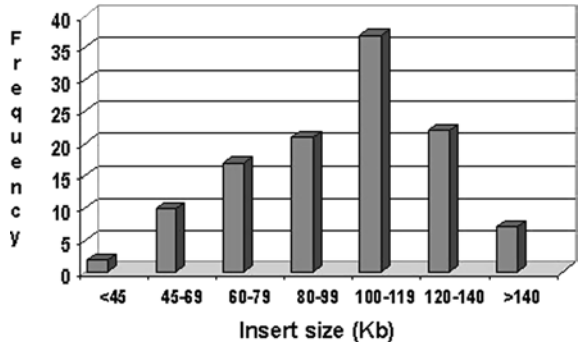

Fig. 2 Insert size distribution of the banana BIBAC library, based on 120 random clones analyzed as Fig. 1

plants were infected using *M. fijiensis*. The EST-TGBR sequence was shown to have little homology with a resistance gene kinase from rice. The EST-HEM sequence was shown to have partial homology with a repressed cDNA fragment homologous to a bitter gourd inhibitor of a sequence-specific *Streptomyces griseus* endopeptidase. (INCO-DC project IC18-CT97-0192; C. Navarro et al. unpublished).

The Tuu Gia PCR product of the RGA9 rice primers (TG-RGC9 fragment) consisted of a single 400-bp band (expected size 452 bp, data not shown); it was sequenced and shown to have little homology with the RGA9 and RGA14 sequence from rice. One part of the translated sequence showed high homology with the motif MQLSLMSLVLGFL, corresponding to both a putative leucine-rich repeat protein kinase, and a receptor kinase-like protein from *Arabidopsis thaliana*. Another membrane copy, prepared from the same microwell plates used with the EST-TGBR probe, was hybridized against the TG-RGC9 fragment, and gave the same hybridization pattern as that of the EST-TGBR (Fig. 4). The Southern hybridization of positive BIBAC clones showed that the EST-TGBR and the TG-RGC9 fragments are located in the same genomic regions (Fig. 5).

Discussion

Highly purified vectors and properly size-selected, high-molecular-weight DNA inserts are essential for the success of BAC and BIBAC library construction, which is consistent with previous studies (Luo et al. 2001). Inclusion of polyvinylpyrolidone in the washing buffer and additional washes for nuclei preparations of banana are important, because polyvinylpyrolidone binds, and

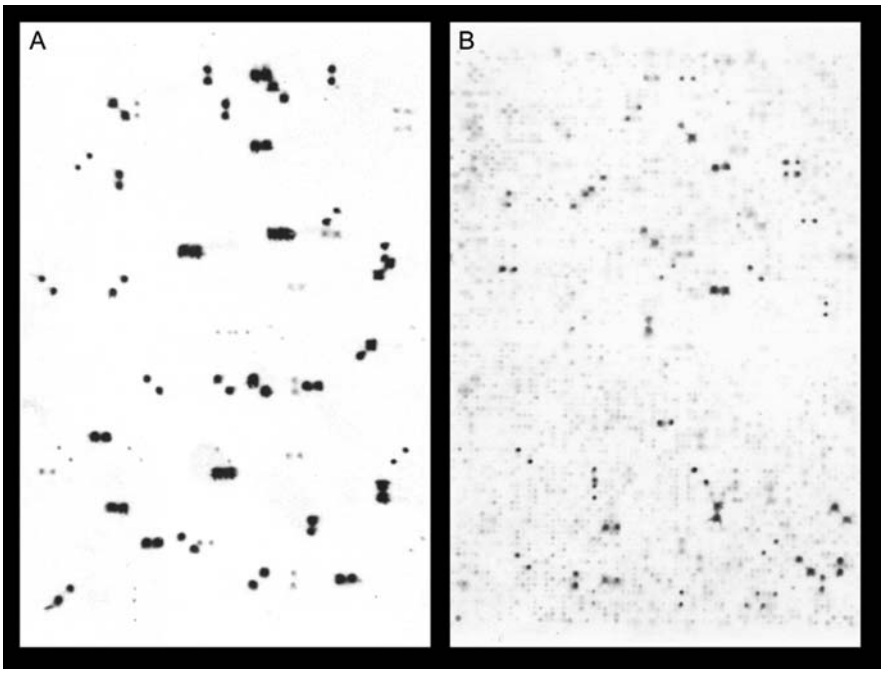

Fig. 3 BIBAC clones containing chloroplast and mitochondrial DNA. Two filters of the BIBAC library (3,072 clones) hybridized with the cpDNA probe made of *psbA*, *ndhA*, and *rbcL* (**a**) and two filters hybridized with mtDNA probes made of *nad4*, *atp*6, and *atp*9 (**b**)

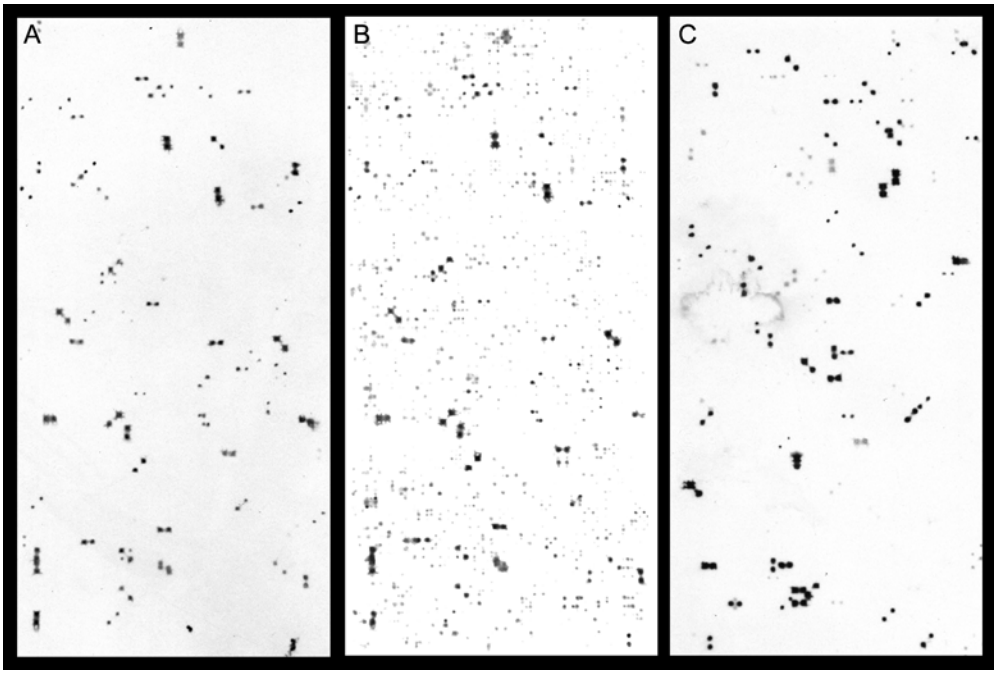

Fig. 4 BIBAC clones identified with the EST-TGBR and the TG-RGC9 fragment, respectively. Three filters of the BIBAC library (4,608 clones) hybridized with the EST-TGBR sequence (**a**), three filters hybridized with TG-RGC9 fragment (**b**), and three filters hybridized with the three chloroplast genes as negative control (**c**)

the additional washes minimize polyphenols that could interact with DNA and thus reduce the quality of DNA fragments.

To reduce the starch levels that can decrease the quality of the plugs, it was adequate to keep the plants in darkness for 48 h before sampling. The quality of agarose plugs is crucial for the successful ligation, the optimal fragment size, and subsequent transformation efficiency. Although double-selection procedures were tested, it was found that using only a single selection with two different runs was sufficient to obtain high transformation efficiency, as Vilarinhos et al. (2003) reported. Moreover, the pre-size selection of the plugs by PFGE before partial digestion eliminated small DNA fragments and further purified the DNA for large fragment cloning, both increasing the cloning efficiency of the library. Another critical point has been the vector: insert molar ratio for ligation. In the present study, the vector:insert molar ratio of 4:1 was shown most desired for BIBAC library construction.

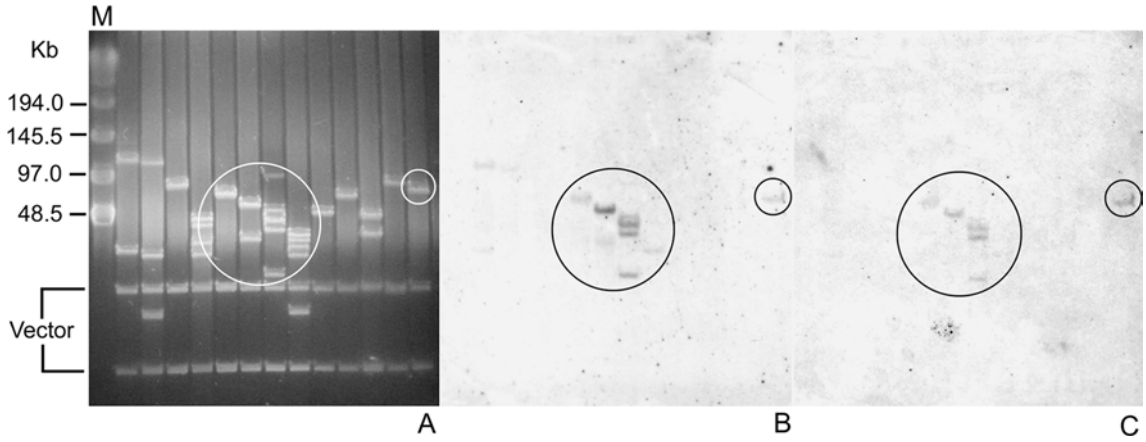

Fig. 5 BIBAC clones analyzed by PFGE and Southern hybridization. (**a**) SYBR Gold-stained gel after PFGE. The BIBAC DNA was digested with *Not*I and separated in a 1% agarose gel. (**b**) Autoradiograph of the blot after hybridization with the EST-

BIBAC libraries have been reported for several species, including tomato, wheat, soybean, *Brassica* spp., *Lotus japonicus*, rice, and *A. thaliana* (Hamilton et al. 1999; Moullet et al. 1999; Meksem et al. 2000; Wu et al. 2000; Men et al. 2001; Tao et al. 2002; Chang et al. 2003), but this paper describes the first BIBAC library for banana (*Musa* spp.). There have already been two BAC libraries of the wild diploid banana *M. acuminata* ssp. *burmannicoides* type Calcutta IV, one BAC library of the triploid *M. acuminata* cv. Grand Nain, and one BAC library of the wild diploid *M. balbisiana* (Vilarinhos et al. 2003; Kaemmer et al. 2002; Safar et al. 2002; INCO-DC project IC18-CT97-0192, A. James et al. unpublished). However, given the nature of BIBAC transformability via *Agrobacterium* in plants (Hamilton et al. 1996, 1999; Liu et al. 1999, 2002; He et al. 2003) and the unique nature of the DNA source, the BIBAC library of *M. acuminata* cv. Tuu Gia (AA) reported here will provide an additional and uniquely powerful tool for banana genome research and breeding, especially functional analysis and genetic engineering of the banana genome.

The fact that 61% of the inserts of the analyzed BIBAC clones contained at least one internal *Not*I site is not surprising. He et al. (2003) reported that there is a higher G+C content in monocotyledonous plants compared to dicotyledonous plants, which increases the chance for a *Not*I recognition site. Therefore, it is difficult to subclone the entire inserts of BAC clones of monocotyledonous plants into a binary vector for genetic transformation (Zhang et al. 1996b). In contrast, all inserts from this pCLD04541-based library are competent for direct transformation mediated by *Agrobacterium*. Stable transformation of inserts shorter than 100 kb has successfully been described in several plant species (Hamilton et al. 1996, 1999; Liu et al. 1999, 2002; He et al. 2003). Although Song et al. (2003) reported that potato DNA fragments larger than 100 kb in BIBAC vectors are not stable in *Agrobacterium*, additional research in this regard is needed, because BIBACs with inserts of larger than 100 kb were shown stable in different *Agrobacterium* strains in several studies (Hamilton et al. 1996, 1999; Liu et al. 1999, 2002; Wu et al. 2000; Men et al. 2002; He et al. 2003). The low number of inserts containing chloroplast DNA (1%) and mitochondrial DNA (0.9%), respectively, is a further demonstration of the high quality of the library.

Depending on the characteristics of particular disease resistance genes in the genus *Musa*, the transfer of preselected clustered resistance gene analogues (RGAs) may assist in the production of various disease-resistant edible banana and plantain cultivars. Although this strategy seems to be questionable in the case of BLS because a major susceptibility gene is possibly present in the genomes of most cultivars, other diseases, e.g., *Fusarium* wilt, can be controlled by a single, dominant resistance gene (Ortiz and Vuylsteke 1994; Vakili 1965).

However, the donor banana of this library, Tuu Gia, is known to possess one of the most effective and durable BLS resistance mechanisms in the *M. acuminata* species (Fullerton and Olsen 1995; Craenen and Ortiz 2003). The responsible battery of highly regulated defense genes is a treasure trove for, among others, inducible promotors, phytoalexin biosynthesis genes, or any kind of signal perception and transduction kinases that are not accessible by *M. fijiensis* molecular attack mechanisms.

Consequently, the first characterization of this BIBAC library included both RGA and EST probes. It is not surprising that NBS-type RGAs hybridize to a high number of BIBAC clones, because the highly conserved nucleotide-binding site is present in many genes coding for kinases or ATP/GTP-dependent enzymes. This number is probably lower, because only 50% of the positive clones could be confirmed when the corresponding BIBAC clones were subjected to Southern analysis. Both ESTs also hybridized to a large number of BIBAC clones, which was unexpected. In the first case, the EST-HEM (which resembles a proteinase inhibitor), the EST expression is down-regulated by fungal extracts.

In the second case, the EST-TGBR, a selected EST candidate turned out to be a possible kinase itself, showing some homology to one of the used RGA sequences from rice. There is some evidence that a few kinases coded by resistance genes can be induced by the pathogen (Yoshimura et al. 1998).

The exactly matching hybridization patterns of the TG-RGC9 fragment and the EST-TGBR are evidence for the clustering of these two genes in close proximity or even their physical identity. It remains that mass transformation of a susceptible banana cultivar should be studied using a mixture of all positive clones identified in this paper, leading to the selection of a single, BLS-resistant cultivar.

According to these results, the analyzed ESTs belong to a group of defense genes that are members of a multigene family. We consider that this library can be used to attempt to recognize clones containing ESTs, as well as family members of resistance gene clusters that are estimated to be up to 100 kb (Hulbert et al. 2001) and eventually to transform banana plants susceptible to BLS. This library is available for the members of the International *Musa* Genomics Consortium.

Acknowledgements We want to thank Dr. Peter M. Gresshoff at the Queensland University, Australia, for his important advises; M.C. Fernando Moguel Salazar, Biol. Caucasella Díaz Trujillo, Q.F.B. Rosa Grijalva, M.C. Margarita Aguilar, and Arqta. Rossana Marrufo at CICY, Merida, Mexico, for their technical support. This work was supported by CONACyT project no. Z-047 and the CoSNET fellowship no. 2001164P.

References

Carreel F, Gonzalez de Leon D, Lagoda P, Lanaud C, Jenny C, Horry J, Tezenas du Montcel H (2002) Ascertaining maternal and paternal lineage within *Musa* by chloroplast and mitochondrial DNA RFLP analyses. Genome 45:679–692

Chang YL, Henriquez X, Preuss D, Copenhaver GP, Zhang, H-B (2003) A plant-transformation—competent BIBAC library from the *Arabidopsis thaliana* Landsberg ecotype for functional and comparative genomics. Theor Appl Genet 106:269–276

Clarke L, Carbon J (1976) A colony bank contained synthetic *Col* E1 hybrid plasmids representative of the entire *E. coli* genome. Cell 9:91–100

Craenen K, Ortiz R (2003) Genetic improvement for a sustainable management of resistance. In: Jacome L, Lepoivre P, Marin D, Ortiz R, Romero R, Escalant JV, (eds) *Mycosphaerella* leaf spot diseases of bananas: present status and outlook. In: Proceedings of the 2nd international workshop on *Mycosphaerella* leaf spot diseases held in San Jose, Costa Rica, 20–23 May 2002. INIBAP Montpellier, France, pp 181–198

Dolezel J, Dolezelova M, Novak FJ (1994) Flow cytometric estimation of nuclear DNA amount in diploid bananas (*Musa acuminata* and *M. balbisiana*). Biol Plant 36:351–357

Fullerton RA, Olsen TL (1995) Pathogenic variability in *Mycosphaerella fijiensis* Morelet, cause of black Sigatoka in banana and plantain. NZ J Crop Hort Sci 23:39–48

Hamilton CM, Frary A, Lewis C, Tanksley SD (1996) Stable transfer of intact high molecular weight DNA into plant chromosomes. Proc Natl Acad Sci USA 93:9975–9979

Hamilton CM, Frary A, Xu Y, Tanksley SD, Zhang H-B (1999) Construction of tomato genomic DNA libraries in a binary-BAC (BIBAC) vector. Plant J 18:223–229

He RF, Wang Y, Shi Z, Ren X, Zhu L, Weng Q, He GC (2003) Construction of a genomic library of wild rice and *Agrobacterium*-mediated transformation of large insert DNA linked to BPH resistance loci. Gene 321:113–121

Hulbert S, Webb CA, Smith S, Sun Q (2001) Resistance gene complexes: evolution and utilization. Annu Rev Phytopathol 39:385–312

INIBAP (2001) Banana food and wealth. International Network for the Improvement of Banana and Plantain, Montpellier, France

Kaemmer D, Peraza-Echeverría L, Canché B, Arroyo A, Ortiz E, Zhang H-B, James A (2002) Characterization and utilization of the first banana BAC library of *Musa acuminata* spp. *burmannicoides* type 'Calcutta IV'. In: Program and abstracts of the 3rd international symposium on molecular and cellular biology of bananas held in Leuven, Belgium, 9–11 September 2002, INIBAP, p 14

Liu Y, Shirano Y, Fukaki H, Yanai Y, Tasaka M, Tabata S, Shibata D (1999) Complementation of plant mutants with large genomic DNA fragments by a transformation-competent artificial chromosome vector accelerates positional cloning. Proc Natl Acad Sci USA 96:6535–6540

Liu YG, Liu H, Chen L, Qiu W, Zhang Q, Wu H, Yang C, Su J, Wang Z, Tian D, Mei M (2002) Development of new transformation-competent artificial chromosome vectors and rice genomic libraries for efficient gene cloning. Gene 282:247–255

Luo M, Wang Y, Frisch D, Joobeur T, Wing RA, Dean RA (2001) Melon artificial chromosome (BAC) library construction using an improved method and identification of clones linked to the locus conferring resistance to melon *Fusarium* wilt (Form-2). Genome 44:154–162

Meksem K, Ruben E, Zobrist K, Hyten D, Tao Q, Zhang H-B, Lighfoot DA (2000). Two large insert soybean genomic libraries constructed in a binary vector: applications in chromosome walking and genome wide physical mapping. Theor Appl Genet 101:747–755

Men A, Meksem K, Kassem MA, Lohar D, Stiller J, Lightfoot D, Gresshoff P (2001) A bacterial artificial chromosome library of *Lotus japonicus* constructed in an *Agrobacterium tumefaciens*-transformable vector. MPMI 14(3):422–425

Moullet O, Zhang H-B, Lagudah ES (1999) Construction and characterization of a large DNA library from the D genome of wheat. Theor Appl Genet 99:305–313

Ortiz R (2000) Understanding the *Musa* genome: an update. Acta Horticult 54:157–168

Ortiz R, Vuylsteke D (1994) Inheritance of black sigatoka resistance in plantain–banana (*Musa* spp.) hybrids. Theor Appl Genet 89:146–152

Perea-Dallos M (1998) Pollen and anther culture in *Musa* spp. In: Galán Súco V (ed) Proceedings of the first international symposium on banana in the subtropics acta horticultural No. 490. Lovaine, Belgium, pp 493–497

Safar J, Piffanelli P, Glaszmann J, Dolezel J (2002) Construction of BAC library for the B genome of banana (*Musa balbisiana*). In: Program and abstracts of the 3rd international symposium on molecular and cellular biology of bananas held in Leuven, Belgium, 9–11 September 2002, INIBAP, pp18–19

Sambrook J, Russell DW (2001) Molecular cloning: a laboratory manual, 3rd edn. Cold Spring Harbor Laboratory Press, Cold Spring Harbor

Song J, Bradeen JM, Naess SK, Helgeson JP, Jinag J (2003) BIBAC and TAC clones containing potato genomic DNA fragments larger than 100 kb are not stable in *Agrobacterium*. Theor Appl Genet 107:958–964

Tao Q, Wang A, Zhang H-B (2002) One large-insert plant-transformation-competent BIBAC library and three BAC libraries of *japonica* rice for genome research in rice and other grasses. Theor Appl Genet 105:1058–1066

Vakili NG (1965) *Fusarium* wilt resistance in seedlings and mature plants of *Musa* species. Phytopatology 55:135–140

Vilarinhos AD, Pifanelli P, Lagoda P, Thibivilliers S, Sabau X, Carrel F, Hont A (2003) Construction and characterization of a bacterial artificial chromosome library of banana (*Musa acuminata* Colla). Theor Appl Genet 106:1102–1106

Wu Y, Tulsieram L, Tao Q, Zhang H-B, Rothstein SJ (2000) A binary vector-based large insert library for *Brassica napus* and identification of clones linked to a fertility restorer locus for Ogura cytoplasmic male sterility (CMS). Genome 43:102–109

Yoshimura S, Yamanouchi U, Katayose Y, Toki S, Wang Z-Y, Kono I, Kurata N, Yano M, Iwata N, Sasaki T (1998). Expression of *Xa1*, a bacterial blight-resistance gene in rice, is induced by bacterial inoculation. Proc Natl Acad Sci USA 95:1663–1668

Zhang H-B, Zhao X, Ding X, Paterson AH, Wing RA (1995) Preparation of megabase-size DNA from plant nuclei. Plant J 7:175–184

Zhang H-B, Woo S-S, Wing RA (1996a) BAC, YAC and Cosmid library construction. In: Foster G, Twell D (eds) Plant gene isolation: principles and practice. Wiley, England, pp 75–99

Zhang H-B, Cho S, Woo S, Li Z, Wing RA (1996b) Construction and characterization of two rice bacterial chromosome libraries from the parents of a permanent recombinant inbreed mapping population. Mol Breed 2:11–24